

CLAIMS

1. A method of regulating the mitotic and/or physiological activities, and differentiation potential of a pluripotent or multipotent cell, which method includes manipulating the expression and/or activity of a cell cycle regulatory molecule including a regulatory molecule selected from one or more of the groups consisting of a cyclin, a cyclin-dependent protein kinase (Cdk), a Cdk inhibitor, upstream regulators thereof or biochemical targets thereof and/or tumour suppressor protein, and molecules displaying similar activities, in a pluripotent or multipotent cell.
- 10 2. A method according to claim 1 wherein the activity of the cell cycle regulatory molecule is manipulated by increasing or decreasing the level of expression of said molecules.
- 15 3. A method according to claim 1 wherein the pluripotent cells or multipotent cells are selected from one or more of a group consisting of epiblast cells, embryonic stem (ES) cells, primitive ectoderm-like (EPL) cells, primordial germ cells (PGCs) or embryonic carcinoma (EC) cells.
- 20 4. A method according to claim 1 wherein the cell cycle regulatory molecule includes a cyclin selected from one or more of the group consisting of cyclin D, cyclin E and cyclin A or a molecule exhibiting similar activity or a functionally active fragment or analogue thereof.
- 25 5. A method according to claim 1 wherein the cell cycle regulatory molecule includes a cyclin-dependent protein kinase (Cdk) selected from one or more of the group consisting of Cdk4, Cdk6 or Cdk2 or a molecule exhibiting similar activity or a functionally active fragment or analogue thereof.
6. A method according to claim 1 wherein the cell cycle regulatory molecule includes a Cdk inhibitor selected from the INK, CIP or KIP families.

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7. A method according to claim 6 wherein the Cdk inhibitor is selected from one or more of the group consisting of p27, p57, p16, p15, p18, p19 or p21 or a molecule exhibiting similar activity or a functionally active fragment or analogue thereof.

5 8. A method according to claim 1 wherein the cell cycle regulatory molecule includes a tumour suppressor protein selected from one or more of the group consisting of retinoblastoma protein (pRb), p107 or p130 or a molecule exhibiting similar activity or a functionally active fragment or analogue thereof.

10 9. A method according to claim 8 wherein the activity or phosphorylation state of the tumour suppressor protein is manipulated.

10. A method according to claim 1 wherein the upstream regulator is selected from the group consisting of one or more of proto-oncogenes *myc* and *ras* and upstream signalling pathways Raf, MAP Kinase or Rho.

15 11. A method according to claim 10 wherein the upstream regulator is *myc* or *ras* and the activity of the regulator is increased.

12. A method for identifying pluripotent or pluripotent-related cells which method includes analysing the cell population for pluripotent cell cycle characteristics including one or more of pluripotent-specific cell cycle structure; pluripotent-specific expression of cell cycle regulatory molecules; and phosphorylation status of a tumour-suppressor protein(s).

13. A method according to claim 12, wherein the method includes measuring expression of cell cycle regulatory molecules, including a cyclin(s), a cyclin-dependent protein kinase (Cdk), a Cdk inhibitor, upstream regulators of said molecules or biochemical targets thereof.

14. A method according to claim 13, wherein the pluripotent or

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pluripotent-related cells are identified by the presence of one or more of the following characteristics:

a pluripotent-specific cell cycle exhibiting a rapid cycle with short gap phases;

5 elevated constitutive expression and/or activity of cyclin E;

elevated constitutive expression and/or activity of cyclin A;

pluripotent-specific expression of Cdk inhibitors; and

presence of a phosphorylated tumour suppressor protein.

15. A method according to claim 14 wherein the pluripotent-specific
10 cell cycle structure is characterised by short periods of the cell cycle in G1 and
G2/M phases with a reduced G2 phase or absence thereof, with a remodelling
of the cell cycle on differentiation.

16. A method according to claim 13 wherein the cyclin E-kinases are
constitutively active at levels more than approximately 50 times those for
15 rapidly dividing primary or transformed somatic cells.

17. A method according to claim 14 wherein the Cdk inhibitors p16
and/or p21 and/or p27 are substantially reduced or absent.

18. A method according to claim 14 wherein the method is used to
identify other cells derived by partial differentiation of pluripotent cells and/or
20 multipotent cells.

19. A method of facilitating maintenance and/or promoting
proliferation of pluripotent cells *in vitro*, said method including
manipulating the expression and/or activity of a cell cycle regulatory
molecule, including a regulatory molecule selected from one or more of the
25 groups consisting of a cyclin, a cyclin-dependent protein kinase (Cdk), a Cdk
inhibitor, upstream regulators thereof or biochemical targets thereof and/or

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tumour suppressor protein, and molecules displaying similar activities, in a pluripotent or multipotent cell such that some or all of the features of the cell cycle of pluripotent cells are enforced.

20. A method according to claim 19 wherein constitutive cyclin E
5 activity and/or constitutive cyclin A activity is increased.

21. A method according to claim 19 wherein constitutive cyclin E
activity and/or cyclin A activity is increased by enforcing expression of
exogenous cyclin E and/or cyclin A activity, and/or enforcing expression of Cdk
2.

10 22. A method according to claim 19 wherein the level of constitutive
cyclin E activity and/or cyclin A activity is increased utilising the proto-
oncogenes *myc* and *ras*.

15 23. A method according to claim 19 wherein constitutive cyclin D
activity is increased by expression of exogenous cyclin D or expression of Cdk
4 and/or Cdk 6.

24. A method according to claim 19 wherein tumour suppressor
proteins are inactivated such that maintenance and proliferation of pluripotent
cells is prolonged.

20 25. A method according to claim 24 wherein the tumour suppressor
protein pRb is inactivated utilising hyperphosphorylation, antisense techniques
or gene inactivation.

26. A method according to claim 19 wherein the upstream regulator
is selected from the group consisting of

25 one or more of proto-oncogenes *myc* and *ras* and upstream
signalling pathways Raf, MAP Kinase or Rho.

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27. A method according to claim 26 wherein the upstream regulator is myc or ras and the activity of the regulator is increased.

28. A method for reprogramming of differentiated or partially differentiated cells to a less differentiated state which method includes

5 manipulating the expression and/or activity of a cell cycle regulatory molecule, including a regulatory molecule selected from one or more of the groups consisting of a cyclin, a cyclin-dependent protein kinase (Cdk), a Cdk inhibitor, upstream regulators thereof or biochemical targets thereof and/or tumour suppressor protein, and molecules displaying similar activities, in said
10 differentiated or partially differentiated cells.

29. A method according to claim 28 wherein the differentiated or partially differentiated cells are reprogrammed to a state of pluripotency.

30. A method according to claim 28 wherein the cell is dedifferentiated to a less differentiated or multipotent state.

15 31. A method according to claim 28 where the cell or nucleus thereof is used as a donor in nuclear transfer.

32. A method according to claim 28 which method includes upregulating cyclin E-Cdk2 and/or cyclin A-Cdk2 activity to direct the differentiated cells or partially differentiated cells towards pluripotency.

20 33. A method according to claim 28 wherein the upstream regulator is selected from the group consisting of one or more of proto-oncogenes *myc* and *ras* and upstream signalling pathways Raf, MAP Kinase or Rho.

34. A method according to claim 33 wherein the upstream regulator is myc or ras and the activity of the regulator is increased.

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25. A method of selecting pluripotent or multipotent cells from a mixed cell population including pluripotent or multipotent cells and differentiated cells which method includes manipulating the expression and/or activity of a cell cycle regulatory molecule, such that the proliferation and maintenance of differentiated cells are reduced, wherein the regulatory molecule is selected from one or more of the groups consisting of a cyclin, a cyclin-dependent protein kinase (Cdk), a Cdk inhibitor, upstream regulators thereof or biochemical targets thereof and/or tumour suppressor protein, and molecules displaying similar activities, in a 10 pluripotent or multipotent cell.
36. A method according to claim 35 which method includes preferentially selecting for the presence or absence of expression of cell cycle regulatory molecules characteristic of a pluripotent cell cycle including elevated cyclin E-associated activities, elevated cyclin A-associated activities and 15 distinct Cdk inhibitor profiles.
37. A method according to claim 36 which method includes enforcing Cdk inhibitor expression and selecting for survival and maintenance of cells exhibiting elevated levels of cyclin E/Cdk2 activity and/or cyclinA/Cdk2 activity.
38. A method according to claim 37 wherein the Cdk inhibitor is a 20 member of the INK family of Cdk inhibitors, or a molecule with similar activity.
39. A method according to claim 38 wherein the Cdk inhibitor is p16.
40. A method according to claim 37 which method includes introducing into said cells a p16 fusion protein or transformation of the cells with a constitutive p16 expression construct and selection for survival and 25 maintenance of cells.
41. A method according to claim 35 wherein the cells selected are

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multipotent cells.

42. A method according to claim 35 wherein the multipotent or pluripotent cells are cells derived by reversion from a differentiated or partially differentiated state to a less differentiated state.

5 43. A method according to claim 42 wherein the reversion is spontaneous or environmentally induced.

44. A method according to claim 35 wherein the cells are in a transitional pluripotent state.

10 45. A method according to claim 42 which method includes initiating ectopic proto-oncogene expression and selecting for cells exhibiting survival and maintenance.

46. A method according to claim 45 wherein the proto-oncogene is *myc*.

15 47. A method according to claim 35 wherein the maintenance and/or proliferation of the selected pluripotent or multipotent cells is facilitated by further manipulating the activity of a cell cycle regulatory molecule.

48. A method of regulating the differentiation of pluripotent or multipotent cells which method includes manipulating the expression and/or activity of a cell cycle regulatory molecule, including a regulatory molecule 20 selected from one or more of the groups consisting of a cyclin, a cyclin-dependent protein kinase (Cdk), a Cdk inhibitor, upstream regulators thereof or biochemical targets thereof and/or tumour suppressor protein, and molecules displaying similar activities, in a pluripotent or multipotent cell, in said cells.

25 49. A method according to claim 48, wherein the differentiation of the pluripotent or multipotent cells is regulated by one or more of the following

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manipulations:

increasing the activity of the tumour suppressor protein;

reducing cyclin E and/or cyclin A-associated activities;

the activity of Cdk2 becomes cell cycle regulated; or

5 upregulating the activities and/or expression of CdK inhibitors.

~~50.~~ A method of regulating the cell cycle of primary or untransformed cells, which method includes

10 manipulating the expression and/or activity of a cell cycle regulatory molecule, such that the proliferation and maintenance of differentiated cells are reduced, wherein the regulatory molecule is selected from one or more of the groups consisting of a cyclin, a cyclin-dependent protein kinase (Cdk), a Cdk inhibitor, upstream regulators thereof or biochemical targets thereof and/or tumour suppressor protein, and molecules displaying similar activities, in a primary or untransformed cell.

15 51. A method according to claim 50 wherein the lifespan of the differentiated or partially differentiated cells is prolonged by one or more of

upregulation of Cyclin E-, Cyclin D- or Cyclin A-associated activities, or upregulation of upstream regulators of cyclin-associated activities.

20 ~~52.~~ A method of identifying differentiating cells in a mixed cell population, which method includes analysing the cell population for differentiating cell cycle characteristics including

differentiation-specific cell cycle structure;

differentiation-specific expression and/or

activity of cell cycle regulatory molecules

25 the presence of active tumour suppressor proteins.

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53. A method according to claim 52 wherein the differentiating cells are identified by the presence of one or more of the following characteristics:

a differentiation-specific cell cycle exhibiting a relatively slow cycle with prominent gap phases; and/or

5 tumour suppressor protein dependency;

 reduced constitutive expression activity of cyclin E and/or cyclin A; and

 upregulated Cdk inhibitor activity, including p16, p21 and/or p27, and

10 Cdk2 activities being cell cycle regulated.

54. Mammalian or avian pluripotent cells whenever produced according to any one of claims 1, 12, 19, 28, 35 and 48.

55. Human pluripotent cells whenever produced according to any one of claims 1, 12, 19, 28, 35 and 48.

15 56. Pluripotent cells that are competent for genetic manipulation whenever prepared according to any one of claims 1, 12, 19, 28, 35 and 48.

57. Multipotent cells and partially differentiated cells whenever produced according to any one of claims 1, 12, 19, 28, 35 and 48.

20 58. Human mammalian or avian multipotent cells and partially differentiated cells whenever produced according to any one of claims 1, 12, 19, 28, 35 and 48.

59. Embryos derived from pluripotent stem cells whenever produced according to any one of claims 1, 12, 19, 28, 35 and 48.

60. Animals derived from embryos according to claim 59.

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61. Genetically modified animals derived from pluripotent stem cells produced according to any one of claims 1, 12, 19, 28, 35 and 48.

62. Use of pluripotent or multipotent cells whenever produced according to any one of claims 1, 12, 19, 28, 35 and 48, in therapies selected
5 from any one of the group consisting of cell therapy, gene therapy, cancer therapy, regeneration and/or development of organs or appendages or limbs, production and/or use in pharmaceuticals and/or diagnostics, xenotransplantation, genetic modification of animals and nuclear transfer.

63. Use of pluripotent or multipotent cells whenever produced
10 according to any one of claims 1, 12, 19, 28, 35 and 48, in drug delivery.